CJES Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Biological and reproductive evidence of invasiveness in silver carp, *Hypophthalmichthys molitrix* from Gheshlagh Reservoir, Kurdistan Province, West Iran

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ABSTRACT

Silver carp, *Hypophthalmichthys molitrix* is one of the most important cultured fish species for human consumption and plays a key role as a natural controller of algal communities in aquatic ecosystems. Basic data on reproductive characteristics such as spawning patterns, absolute and relative fecundity, and their relationships with age, body length, weight, sex ratio, egg diameter, and length at the first sexual maturity are essential for effective fisheries management. This study examined biological and reproductive parameters of silver carp in Gheshlagh Dam, Sanandaj City, West Iran across four sampling periods from September to December 2021. Fish were collected from two stations in Gheshlagh reservoir, and reproductive indices were analyzed in the laboratory. The population comprised four age groups (3–6 years), with mean weights ranging from 852.5 ± 103.9 g to 2216.5 ± 450.43 g (overall mean: 1130.4 ± 384.4 g; n = 38). Mean gonadosomatic index (GSI) values were 4.1 and 4.8 for 3 and 4-year-old fish, and 5.1 and 5.5 for 5- and 6-year-olds, respectively. Average condition factor (CF) values were 0.96 and 0.94 for 3- and 4-year-olds, and 1.02 and 1.04 for 5- and 6-year-olds. Evaluations of gonadal maturity showed that environmental conditions in Gheshlagh Dam are unfavorable for full gonadal development and most likely do not allow for natural spermatogenesis and spawning of this species. Given their inability to reproduce and their filter-feeding diet, silver carp can serve as an effective biological control agent for algal communities in freshwater reservoirs. Therefore, it is unscientific to classify them as an invasive species in Gheshlagh Dam and similar reservoirs.

Keywords: Biological parameters, stock assessment, reproductive indices, Gheshlagh Dam.

INTRODUCTION

Understanding fish in aquatic ecosystems is crucial for ecology, conservation, stock assessment, and fishery management. Aquatic ecosystems face severe water stress from global warming and human activity, threatening fish survival, especially that of commercial species. Studying fish biology and ecology can help decision-makers conserve and sustainably utilize these stocks. Key to conservation is determining fish reproductive and spawning periods through sexual stages, gonadal index, and fecundity, all vital aspects of fish reproductive biology (Biswas 1993; Larigany *et al*. 2023).

Reproduction is a set of interconnected processes that begin with the growth and development of the gonads under the influence of external and internal factors, continue with the development of secondary sexual characteristics, and ultimately lead to the production of gametes, that are responsible for transmitting genetic information to the next generation. The ultimate goal of all the above processes is to conserve the species and ensure its survival (~~Falahetkar~~ Falahatkar & Javid Rahmdel 2010).

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Reproduction is an important part of an animal's life. Fish are the most diverse group of vertebrates and, as a result, have a wide variety of reproductive methods (Desjardins & Fernald 2009). Environmental factors such as temperature and photoperiod (light period) play an important role in the growth and sexual maturation of fish (Quintana *et al.* 2004). Despite the fact that silver carp has been introduced in many countries of the world for biological control (algal blooms) and aquaculture, natural reproduction is observed only in limited locations in ecosystems similar to the original environment (Kolar *et al.* 2005). Silver carp's natural reproduction is dependent on geographical conditions and food availability (Nazari 1996). Sexual maturity of silver carp occurs earlier in tropical regions than in temperate and cold regions, depending on the ambient temperature (Nazari 1996). Silver carp show variable spawning habits, as it has been reported that in the Amur River, they spawn between June and early August (Gorbach & Krykhtin 1989).

Hypophthalmichthys molitrix, commonly known as silver carp, belongs to the class Osteichthyes (bony fishes), order Cypriniformes, family Xenocyprididae, subfamily Hypophthalmichthyinae, and genus *Hypophthalmichthys* (Eagderi *et al.* 2022), and it is distributed across nearly all continents (Banarescu & Coad 1991).

Their typical size ranges from 45 to 55 cm, with a maximum length of 105 cm and weight up to 12 kg (Vasile 2007). This fish has a single row of pharyngeal teeth arranged 4–4, as well as long gill rakers and fused gill filaments that form a mesh-like filtering surface. It has a small, upward-facing mouth, which is well adapted for feeding on algal and phytoplanktonic communities in the water (Vossoughi & Mostajir 2000).

Silver carp is native to a range that extends from the Amur River in the North to the Pearl River in southern China (Kolar *et al.* 2005). It is cultivated in polyculture systems throughout Asia and Europe (Opuszynski, 1979; Rimon & Shilo 1982). Additionally, this species is stocked in lakes and reservoirs to enhance fish production (Barthelmes & Kleibs 1978). Eutrophication and algal blooms in freshwater sources have raised concerns regarding drinking water safety (Wert *et al.* 2013; Weirich *et al.* 2014). Previous efforts to control algal growth have mainly focused on two approaches: the use of herbicides and the reduction of nutrient inputs (Anonymous 1975). However, in addition to the costs and environmental risks associated with herbicide use (Janik *et al.* 1980), such methods may also have counterproductive effects, as herbicides can often stimulate phytoplankton activity instead of suppressing it (Tucker & Boyd 1978). In addition to chemical (Chen & Yeh 2005) and physical methods (Borchardt & Omelia, 1961; Bare *et al.* 1975; Babel *et al.* 2002) for removing phytoplankton in water treatment processes, biological control of phytoplankton in large aquatic ecosystems, such as lakes, is also effective (Laws & Weisburd, 1990; Carpenter *et al.* 1995). Previous studies have examined the impact of filter-feeding fish, particularly silver carp, on the structure of plankton communities and their potential use as a biomanipulation technique to reduce algal biomass (Starling & Rocha 1990; Starling 1998). Recently, the use of silver carp with a filter-feeding diet has become a direct biomanipulation method for controlling algal blooms. Biological control by silver carp in eutrophic water ecosystems has been widely applied to manage phytoplankton and improve water quality (Radke & Kahl 2002; Kaihong *et al.* 2002; Ma *et al.* 2009). Numerous studies have been conducted in this area, and their results clearly demonstrate the positive effects of filter-feeding fish, such as silver carp, on water quality (Fukushima *et al.* 1999; Kaihong *et al.* 2002; Xiao *et al.* 2010).

Therefore, considering that the silver carp species has the ability to filter-feed and thereby manage algal blooms, studying its reproductive characteristics and understanding its breeding conditions in the Gheshlagh aquatic system can provide an effective strategy to increase the population of this species. This, in turn, can help reduce and control algal blooms in this ecosystem, which serves as a drinking water source for Sanandaj City, making it of great importance.

MATERIALS AND METHODS

Sampling: The Gheshlagh (Vahdat) Reservoir Dam in Sanandaj City is located on the Gheshlagh River, nestled between the mountains of Satileh and Tirgaran, approximately 13 km from Sanandaj towards Divandareh City. The dam reservoir was filled in 1979. The lake behind the dam is 11 km long, with a capacity of about 220 million m^3, an area of approximately 950 hectares, and a maximum depth of around 60 m at the normal water level (Fig. 1). For sampling fish and water, two stations were selected based on the study objectives. Station 1 (eastern side of the dam) and Station 2 (western side) are located within the reservoir body (Table 1).

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Fig. 1. Gheshlagh Dam (Vahdat) in Sananndaj City.

Table 1. Sampling stations coordinates.

Coordinates	Station
35° 26' 50.5"N, 47° 00' 11.9" E	St. 1
35° 26' 25.5"N, 46° 59' 3.00" E	St. 2

Fish sampling was conducted bimonthly from September to March 2021 at two stations located in the main body of the Gheshlagh Dam. Coordination was made with the Gheshlagh Dam fishing cooperative in Sanandaj City to collect specimens and examine the diet of silver carp. The cooperative's sampling method involved the use of gillnets with mesh sizes of 60, 65, and 70 mm. Simultaneously with fish sampling, water samples were also collected from Stations 1 and 2. For this purpose, 1-L samples were taken from the photic zone of the reservoir and preserved using 4% formalin (Fig. 2).

Fig. 2. Sampling of silver carp from Gheshlagh Dam.

Biometric analysis of the captured specimens was conducted immediately. Biological variables such as total length and weight of the fish were measured at the Gheshlagh Dam fishing cooperative station using a measuring board and a digital scale with precisions of 1 mm and 1 g, respectively. Subsequently, the viscera samples were transported to the Fisheries Department Laboratory, University of Kurdistan, where they were weighed using a scale with 0.01 g precision and preserved in 10% formalin (Fig. 3). Additional variables such as age determination (based on scales), sex determination, and gonadal maturation stage (for female fish) were also determined.

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Fig.3. Biometric measurements, dissection, and gonadal tissue separation of silver carp.

Gastrosomatic Index (GaSI): This index is used to estimate the feeding intensity of fish and is calculated based on the following formula (Desai 1970):

$$\mathrm{Ga.SI} = \frac{\text{Weight of gut (Wg)}}{\text{Total body weight (BW)}} \times 100$$

Condition Factor (CF): The condition factor indicates the biological condition of fish (Saborowski & Buchholz 1996). A higher CF value suggests better biological condition and more energy allocated to fish growth (Biswas 1993). The index is calculated using the following formula:

$$\mathrm{CF} = \frac{100\mathrm{W}}{L^3}$$

where CF is the condition factor, W is the total body weight (g), and L is the total length (cm).

If $0.2 < \mathrm{CF} < 0.3$, it indicates poor or very poor condition; if $0.4 < \mathrm{CF} < 0.5$, it indicates moderate condition; and if $\mathrm{CF} > 0.5$, it indicates a good condition regarding fish plumpness (Saborowski & Buchholz, 1996).

Hepatosomatic index (HSI): The hepatosomatic index is used to assess the reproductive cycle and nutritional status of fish. It is calculated using the following formula (Biswas, 1993):

$$\mathrm{HSI} = \frac{\text{Liver weight (Wg)}}{\mathit{Total\ Body\ weight\ (Bw)}} \times 100$$

Gonadosomatic index (GSI): The gonadosomatic index indicates the sexual maturity status of fish. It is calculated based on the ratio of gonad weight to total body weight using the following formula (Biswas 1993):

$$\mathrm{GSI} = \frac{\text{Gonad weight}}{\mathit{Total\ Body\ weight\ (Bw)}} \times 100$$

Histological method of gonadal tissue in silver carp: To investigate histological changes in the gonadal tissue of silver carp from Gheshlagh Dam Lake, a total of 10 specimens of both sexes (male and female) were caught during the autumn and winter seasons. After fixing the gonadal tissue in a 10% formalin solution, samples were

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taken from the middle part of the gonad. The processes of dehydration, clearing, paraffin infiltration, and embedding were carried out according to the method described by Sadouni & Iguer-Ouada (2019). Subsequently, 5 µm thick sections were prepared, stained with haematoxylin-eosin, and the stages of gonadal growth and development were described based on the descriptive method of Sadouni & Iguer-Ouada (2019).

The collected data were entered into Excel 2016 and after preliminary analysis, the necessary graphs were plotted. First, the normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Then, comparisons of the mean values of the measured indices between the two stations were performed using the independent t-test. Differences across various sampling periods were analyzed using one-way ANOVA, followed by Duncan's test for multiple comparisons, all with a 95% confidence interval. These analyses were conducted using SPSS version 21.

RESULTS

The biometric characteristics of the examined silver carp from Gheshlagh Dam are presented in Table 2. Based on the results, four age groups (3 to 6 years) were identified during the study period. The mean weight of the age groups ranged from 852.5±103.9 g to 2216.5 ± 450.43 g. A significant difference was observed between the 6-year age group and the other groups ($p < 0.05$), while no significant difference was found between the 3- and 4-year groups or between the 4- and 5-year groups.

With increasing age, the total length varied from 445.11 ± 19.12 mm to 595.50 ± 48.79 mm. The differences between age groups were statistically significant ($p < 0.05$), except between the 4- and 5-year-old groups. The gut weight of 3-year-old fish was approximately 54.73 ± 24.21 g, which increased with age, reaching around 157.76 ± 28.17 g in 6-year-old specimens (Table 2).

Table 2. Mean (± standard deviation) of biometric characteristics of silver carp, *Hypophthalmichthys molitrix* in the Gheshlagh Dam (Weight in g, Length in mm).

Liver weight (g)	GI tract weight (g)	Total length (mm)	Fish weight (g)	Age (Year)
8.1 ± 2.9^{c}	54.7 ± 24.2^{b}	445.11 ± 19.12^{c}	852.5 ± 103.9^{c}	3+
9.7 ± 2.6^{bc}	90.24 ± 36.06^{b}	479.2 ± 33^{bc}	1044.2 ± 221.8^{bc}	4+
13 ± 3.05^{b}	129.6 ± 36.2^{a}	498.06 ± 31.6^{b}	1258.6 ± 316.4^{b}	5+
23.5 ± 0.8^{a}	157.8 ± 28.2^{a}	595.5 ± 48.8^{a}	2216.5 ± 450.4^{a}	6+

Note: Superscript letters (a, b, and c) indicate significant differences at $p < 0.05$.

The mean Gastrosomatic index (GaSI) at ages 3 and 4 was 1.4 and 4.8, respectively, and at ages 5 and 6 it was 5.1 and 5.5, respectively. The mean Condition Factor (CF) for the examined samples was 0.96 and 0.94 at ages 3 and 4, as well as 1.02 and 1.04 at ages 5 and 6, respectively. Additionally, the mean hepatosomatic index (HSI) for the 3- and 4-year-old groups was 0.94 and 0.93, respectively, and for the 5- and 6-year-old groups it was 1.01 and 1.6, respectively. A significant difference ($p < 0.05$) was observed between the 6-year-old group and the other age groups (Table 3).

Table 3. Mean (± standard deviation) of biological indices of silver carp, *Hypophthalmichthys molitrix* in the Gheshlagh Dam.

Age (Years)	Number of Fish	GaSI (Gastrosomatic Index)	CF (Condition Factor)	HSI (Hepatosomatic Index)
3+	9	4.1 ± 1.04^{c}	0.96 ± 0.04^{bc}	0.94 ± 0.24^{b}
4+	16	4.8 ± 1.6^{b}	0.94 ± 0.05^{c}	0.93 ± 0.22^{b}
5+	11	5.1 ± 1.7^{b}	1.02 ± 0.07^{ab}	1.01 ± 0.08^{b}
6+	2	5.5 ± 1.9^{a}	1.04 ± 0.04^{a}	1.6 ± 0.57^{a}

Note: Different superscript letters (a, b, and c) indicate significant differences at $p < 0.05$.

To investigate the reproductive status of silver carp in Gheshlagh Dam, several specimens of both male and female fish were examined. The range of results for macroscopic and microscopic indices is presented in Table 4. In the macroscopic examination of gonadal tissue during the study period, most gonads showed no significant development, appearing as a pair of thin, thread-like filaments in the majority of individuals (Fig. 4). Histological

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analysis of the gonads was performed on specimens collected from September to March, following the identification key by Sadouni & Iguer-Ouada (2019). Most testicular tissue samples contained spermatogonia cells, and in some lobules, primary and secondary spermatocytes were also observed (Fig. 5). The diameter of spermatogonia ranged from 2.5 to 3.75 µm, and primary spermatocytes measured 10 to 17.5 µm in diameter. Based on the size and morphology of germ cells in the testicular tissue, it appears that male fish were at sexual maturation stages 1 to 2 during the study period. The macroscopic observations and gonadosomatic index (GSI) values of male fish also indicated a lack of testicular development.

Table 4. Range of macroscopic and microscopic reproductive indices in male and female silver carp, *Hypophthalmichthys molitrix* from the Gheshlagh Dam Lake.

Sex	Age (Years)	Gonad Weight (g)	Body Weight (g)	Gonadosomatic Index (GSI %) Range	Maturity Stage Based on Histological Key	Oocyte Diameter (µm)
Male	3–6	0 – 3.01	992 – 1800	0.12 – 3.37	1 – 2	–
Female	3–6	2.2 – 115.58	760 – 2535	0.12 – 4.56	1 – 3	66 – 1145

Microscopic examination of ovarian tissue sections of silver carp from September to March revealed that in the samples collected in September (marking the end of the favourable breeding season for warm-water fish), the ovarian tissue contained primary oocytes with diameters ranging from 860 to 1145 µm, and both the zona radiata and follicular layers were distinguishable (Fig. 6). Macroscopic examination of some female fish during this month showed ovarian tissue containing large cells with yolk reserves and numerous degenerated oocytes appearing white in colour (Fig. 7). In other months, most sections contained oogonia cells with dense and homogeneous cytoplasm (Fig. 8). These cells exhibited irregular shapes with multiple nucleoli and measured between 66 and 156 µm in diameter.

Fig. 4. The photograph of testis in a silver carp from Gheshlagh Dam Lake, weighing 1102 grams, caught in September.

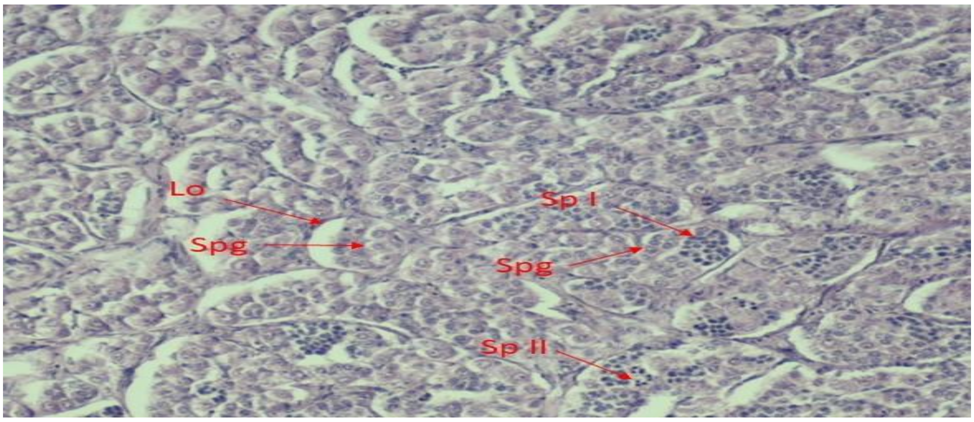

Fig. 5. Microscopic section of testis tissue of silver carp from Gheshlagh Dam Lake. **LO**: Lobule, **Spg**: Spermatogonia, **Sp I**: Primary spermatocyte, **Sp II**: Secondary spermatocyte (× 200).

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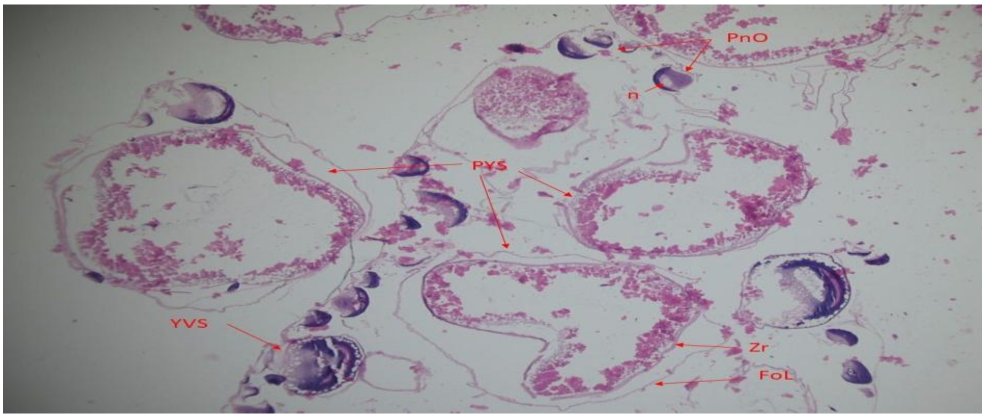

Fig. 6. Cross-section of ovarian tissue in female silver carp samples from Gheshlagh Dam Lake, collected in September; **PnO**: Stage I (oocyte with peripheral nucleus), **YVS**: Stage II (yolk vesicle stage), **PYS**: Stage III (primary yolk synthesis), **n**: nucleus, **Zr**: zona radiata, **Fol**: follicular layers (× 40).

Fig. 7. Ovary of a silver carp caught in Gheshlagh Dam Lake, during September. The ovary weighs 27.7 g and belongs to a 1502 g- female, containing a large number of white (degenerated) eggs.

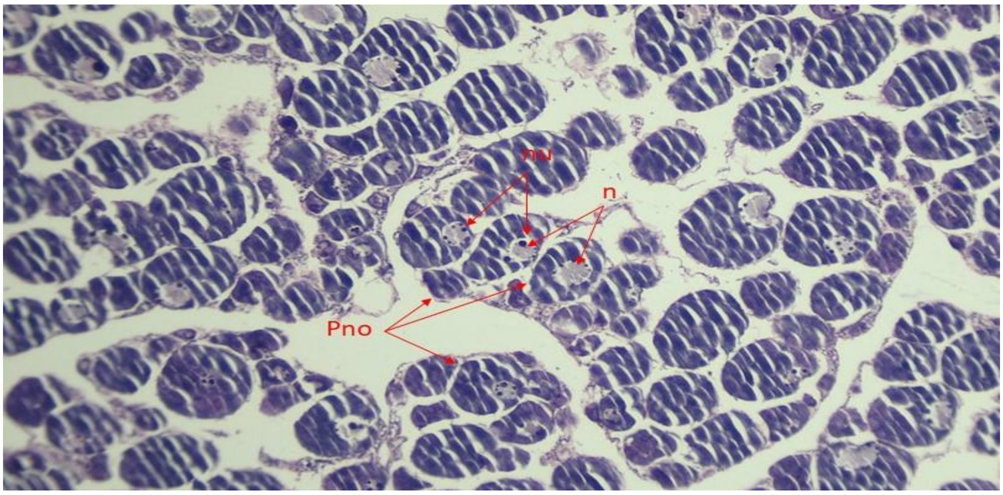

Fig. 8. Ovary tissue of silver carp during autumn and winter containing numerous oocytes at the perinucleolar stage (stage one) of sexual maturation. **PnO**: perinucleolar oocyte, **n**: oocyte nucleus, **nu**: nucleolus (×100).

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DISCUSSION

This study was conducted to investigate the reproductive biology of silver carp, *Hypophthalmichthys molitrix* in Gheshlagh Dam, Sanandaj, western Iran. Based on the results, four age groups ranging from 3 to 6 years were identified. In Lake Chitgar, Tehran, the age of the fish was determined to range from 1 to 7 years (Abbasi *et al.* 2022). Additionally, in a study of 142 silver carp specimens from Lake Tudakul, Uzbekistan, the age range was reported to be from 1 to 5 years (Kamilov 2014). In another study of 145 silver carp specimens in the Mississippi River, six age groups ranging from 0 to 5 years were identified (Williamson *et al.* 2005).

Differences in the age composition observed across these studies can be attributed to several factors, including variations in fishing methods, net mesh sizes, and the absence of older fish due to harvesting in previous years. In the present study, gillnets with mesh sizes of 60, 65, and 70 mm were used to catch fish of commercial size. In contrast, Abbasi *et al.* (2022), employed gillnets with finer mesh sizes (15 to 100 mm) and beach seine nets with a mesh size of 6 mm. In Kamilov's study, gillnets with mesh sizes ranging from 24 to 120 mm were used (Kamilov 2014).

The mean gastrosomatic index (GaSI) of silver carp in Gheshlagh Dam was recorded as 1.4 and 4.8 for age classes 3^+ and 4^+ years, respectively, and 5.1 and 5.5 for age classes 5 and 6 years, respectively. In comparison, the GSI values for silver carp in Kaygaon Toka, India, ranged between 0.453 and 1.37 (Chhaya & Ravi 2022). Mogodan *et al.* (2021) study in aquaculture farms in Romania, reported the GaSI for silver carp as 1.98 ± 0.47. The gastrosomatic index is a well-established indicator of feeding intensity and nutritional condition in fish (Desai 1970). The relatively higher GaSI values observed in the silver carp population of Gheshlagh Dam may reflect favourable feeding conditions in this habitat.

The mean condition factor (CF) of silver carp specimens from Gheshlagh Dam was calculated as 0.96 and 0.94 at ages 3^+ and 4^+ years, as well as 1.02 and 1.04 at ages 5 and 6 years, respectively. In comparison, the average CF values for silver carp in Lake Taihu, China, were reported as 2.27 and 2.23 in 2004 and 2005, respectively (Ke *et al.* 2008). Results from an assessment of 469 silver carp across three rivers in the United States indicated that the highest CF value, 1.8, occurred during the spring season (Hayer *et al.* 2014). In Lake Flag Boshielo, South Africa, the average CF of 87 silver carp specimens was reported as 1.30 (Lubcker *et al.* 2016). Rezaeetabar *et al.* (2017) worked on warm-water fish ponds in Rasht City, northern Iran, reporting that CF values for silver carp in June, August, and October were 1.06, 1.18, and 1.12, respectively. Additionally, in Khorramshahr warm-water aquaculture farms, South Iran, the CF at the end of the rearing period was determined to be 1.56 (Jafarian *et al.* 2019).

Overall, the CF values observed in the present study are largely consistent with those reported in previous studies, with only slight variations. These minor differences may be attributed to varying environmental conditions, differences in feeding regimes, and variation in fish sizes across the studied populations.

Macroscopic and microscopic examination of gonadal tissue in silver carp from Gheshlagh Dam revealed that the testicular tissue in all examined male specimens was in the early stages of gonadal development, characterized as immature or developing testes. In females, the ovarian tissue was predominantly in the stages containing primary oocytes, cortical alveoli formation, and early vitellogenesis. However, in the examined testicular sections, lobule size was small, and the density of germ cells was low. In some female specimens collected in September, a considerable number of degenerated oocytes were also observed in the ovarian tissue.

Previous studies on the natural reproduction of silver carp populations in various parts of the world have demonstrated that, despite the advancement of gonadal maturation, natural spawning in this species often does not occur (Sadouni & Iguer-Ouada 2019). Although silver carp has been widely introduced in many countries for biocontrol of algal blooms and aquaculture purposes, natural reproduction has only been documented in a few areas with environmental conditions similar to their native habitats (Kolar *et al.* 2005).

The results of the present study suggest that environmental conditions in Gheshlagh reservoir may be suboptimal for full development of testicular and ovarian tissues in silver carp, likely preventing successful natural spawning and spermiation. However, confirmation of this hypothesis requires further detailed investigations.

Phytoplankton (algae) constitute the primary food source for silver carp, and this species is well adapted to efficiently utilize these biological communities for nourishment. Silver carp also exhibits selective feeding behaviour, often consuming the most abundant food items available in the environment. With its filter-feeding diet, silver carp has the potential for bioremediation by effectively reducing algal biomass, making it a viable

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method for controlling algal blooms without adverse environmental consequences. Despite its ecological benefits, several studies on silver carp populations across different regions of the world have shown that, although the species can reach advanced stages of gonadal development, natural spawning often does not occur. The findings of the present study suggest that environmental conditions in Gheshlagh Dam are likely unsuitable for the full development of gonadal tissues in silver carp, indicating a limited potential for natural reproduction of the species in this habitat.

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Caspian J. Environ. Sci.
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